During the interview of August 6, 2001, at which the Examiner, Dr. Kerr, the SPE, Dr. Clark, co-inventor, Dr. Prockop, and counsel for Applicants, Dr. Kathryn Doyle and the undersigned, were in attendance, it was agreed that, with regard to the 35 U.S.C. §112, enablement rejection, Applicants would be allowed to establish, by way of a declaration pursuant to 37 C.F.R. §1.132, that MSC-based cell and/or gene therapy is/are effective in treating a CNS disease in a suitable animal model.

Further, with regard to the 35 U.S.C. §103(a), obviousness rejection, it was determined that the rejection was based on the combination of Caplan (1991, J. Orthopaedic Research 9:641-650) ("Caplan"), taken with Pereira et al. (1995, Proc. Natl. Acad. Sci. USA 92:4857-4861) ("Pereira"), Friedmann (1994, TIG 10:210-214) ("Friedmann"), and Prockop (1997, Science 276:71-74). That is, Eglitis et al. (1997, Proc. Natl. Acad. Sci. USA 94:4080-4085), was apparently cited inadvertently instead of Caplan. In addressing the obviousness rejection based on Caplan, Pereira, Friedmann, and Prockop, it was agreed that Applicants would present arguments asserting that this combination of references does not suggest or make obvious differentiating stromal cells *in vitro* to the co-cultured cell phenotype.

In accordance with the agreement memorialized in the Interview Summary of August 6, 2001, Applicants aver as follows.

Rejection of Claims 1-18, Under 35 U.S.C. § 112, first paragraph

Claims 1-18 stand rejected under 35 U.S.C. § 112, first paragraph, because in the Examiner's opinion, cell and gene therapy using marrow stromal cells are not enabled by the disclosure in the specification given the unpredictability of the art at the time of filing. Applicants have previously addressed this rejection in Responses to previous Office Actions, which Responses were filed on February 10, 2000 (responding to Office Action mailed October 4, 1999; Paper No. 6), and November 20, 2000 (responding to Office Action mailed May 24, 2000; Paper No. 12). Applicants hereby incorporate by reference the arguments set forth in those Responses as if set forth in their entirety herein.

In accordance with the agreement reached at the Interview of August 6, 2001, Applicants have amended the claims to recite that the CNS diseases, disorders or conditions are selected from the group consisting of Parkinson's disease, stroke, and spinal cord injury. These amendments are supported throughout the specification as filed, commencing on page 16, lines

23-26. More particularly, the specification as filed makes clear that the invention encompasses "treatment of Parkinson's disease, Alzheimer's disease, spinal cord injury, stroke ...." (specification at page 16, lines 24-25). Thus, no new matter has been added by way of this amendment. Based upon the disclosure provided in the specification, and as demonstrated by the subsequent reduction to practice according to the teachings provided therein, it is clear that one skilled in the art would have been able to treat Parkinson's disease, spinal cord injury and stroke without undue experimentation as more fully set forth below.

Applicants respectfully submit the Declaration of co-inventor, Darwin J. Prockop, pursuant to 37 C.F.R. §1.132 (hereinafter referred to as "the Declaration"), to support that, at the very least, the specification as filed supports claims reciting treatment of several human CNS diseases, disorders, or conditions, *e.g.*, Parkinson's disease ("PD"), stroke, cerebral ischemia, and spinal cord injury, as demonstrated in art-recognized models for these diseases, disorders and conditions.

That is, the data set forth in the declaration demonstrate that since the specification was filed, the invention has been further reduced to practice by Dr. Prockop and by others following the teachings of the invention, demonstrating the use of marrow stromal cells (MSCs) for cell and gene therapy treatment of central nervous system (CNS) diseases, disorders and conditions. The references cited in the declaration, except for the unpublished thesis of Emily Schwarz, are cited in the Supplemental Information Disclosure Statement and attendant Form PTO-1449, which accompany this Preliminary Amendment.

More specifically, the declaration discloses that Dr. Prockop and co-inventor S. Ausim Azizi, have obtained data which demonstrate that recombinant MSCs engraft the mammalian CNS and provide therapeutic benefit in an art recognized model of CNS disease, *i.e.*, Parkinson's disease (PD). This data is disclosed in Schwarz et al., 1999, Human Gene Therapy 10:2539-2549, a copy of which is attached to the declaration as Exhibit "A." Briefly, human and rat MSCs were transduced using a retrovirus vector such that the cells express enzymes necessary for synthesis of L-DOPA, *i.e.*, tyrosine hydroxylase (TH, rate-limiting enzyme in dopamine synthesis) and GTP cyclohydrolase I (GC, enzyme necessary for synthesis of tetrahydrobiopter cofactor, BH_4, for TH). The transduced cells were engrafted into rats lesioned using 6-OHDA (6-hydroxydopamine), which is an art recognized model of human Parkinson's disease.

Schwarz et al., 1999, demonstrates that donor cells were present in the recipient brains at 87 days post-engraftment and that the cells had migrated extensively throughout the brain. Moreover, although the proteins were expressed for 14 days due to transgene shutdown known to occur with this viral vector, the data demonstrate that L-DOPA and metabolites thereof were present in the engrafted brains (Exhibit "A", Figs. 3A-B), and the recipient rats demonstrated behavioral recovery (*e.g.*, decreased rate of apomorphine-induced rotation) (Exhibit "A" at page 2543, Fig. 2).

More recently, data from Dr. Prockop's laboratory, now disclosed in Chapter 5 of the unpublished thesis of doctoral candidate Emily J. Schwarz, pages 102 to 121 (attached to the declaration as Exhibit "B"), demonstrate that rat MSCs (rMSCs) transduced using self-inactivating retrovirus vectors expressing TH and GC downstream of PGK promoter (rMSC-pSIR-PGK-TIG) and transplanted into lesioned rat brains, in an art accepted murine model of PD, mediate behavioral correction (*e.g.*, decreased rate of apomorphine-induced rotation) compared with lesioned rats transplanted with control MSCs, *i.e.*, transduced with a non-specific (green fluorescence protein) GFP gene (Exhibit "B," page 109, Fig. 27).

Additionally, the data disclosed herein demonstrate production of L-DOPA and metabolites thereof in donor rat brains following transplant with transduced MSCs expressing the L-DOPA synthesis enzymes (*id.* at page 111, Fig. 28). Furthermore, the data disclosed demonstrate that the MSCs preferentially migrate to damage sites and along needle tracks caused by the technique to produce the lesion (*id.* at page 116, Fig. 31). Moreover, the data disclosed in the declaration demonstrate transplanted MSCs differentiate into neurons (expressing neuronal marker NeuN) (*id.* at page 120, Fig. 33).

Therefore, Schwarz et al., 1999 (Exhibit "A"), and Schwarz et al., unpublished thesis (Exhibit "B"), demonstrate successful MSC gene therapy whereby an exogenous nucleic acid encoding a beneficial protein was introduced into and expressed in MSCs and the recombinant cells were the administered to a recipient thereby effecting treatment of a disease, disorder or condition of the CNS using an art-recognized model of such disease, disorder or condition. The data set forth in Exhibits "A" and "B" clearly demonstrate that MSCs can be genetically engineered and used successfully to effect gene therapy comprising expression of a therapeutic protein to treat a CNS disease, disorder or condition as disclosed in the specification as filed.

Furthermore, these gene therapy data obtained in an art-recognized model of Parkinson's disease correlate with a reasonable expectation of successful treatment of human disease since it is well known in the art that results obtained using such animal models are likely to be applicable to treatment of human subjects. Therefore, with respect to claims 1-8, reciting gene therapy methods using MSCs expressing an exogenous nucleic acid encoding a therapeutic protein, the data presented herein disclosing the data of Schwarz et al., firmly supports those claims. Thus, the data provided herein demonstrate the further reduction to practice of the invention according to the teachings of the specification as filed in that gene therapy using MSCs to treat a disease, disorder, or condition of the CNS, has been amply demonstrated for Parkinson's disease.

With regard to using MSC cell therapy to treat human disease, disorder or condition, where the cells are obtained from a normal donor and administered to a recipient without recombinant manipulation, the declaration sets forth, *inter alia*, the data disclosed in Horwitz et al., 1999, Nature Med. 5:309-313. Horwitz et al., 1999, on which Dr. Prockop is a co-author, is attached to the declaration as Exhibit "C". Horwitz et al., have further reduced the invention to practice, demonstrating that MSC cell therapy methods, which were first developed using non-human animal studies demonstrating treatment of osteogenesis imperfecta (OI) in mice, can be successfully applied to treatment of humans.

More specifically, Dr. Prockop and his colleagues, in Pereira et al. (1998, Proc. Natl. Acad. Sci. USA 95:1142-1147), had previously demonstrated that MSC cell therapy was effective in treating mice in an art-recognized model of osteogenesis imperfecta. In Horwitz et al., 1999, Dr. Prockop and his colleagues demonstrated that the teachings of the art recognized animal model could be successfully applied to treat humans. That is, MSCs from normal HLA-identical or single-antigen-mismatched siblings were transplanted into children with severe deforming osteogenesis imperfecta thereby treating the disease.

The data disclosed in Horwitz et al., 1999, demonstrate that only as little as approximately 1.5-2% donor MSCs (determined by detecting Y chromosome in osteoblasts or DNA polymorphism in osteoblast of same-sex match) mediated clinically detectable improvement in various clinical parameters for assessing the severity of OI and the effectiveness of any treatment thereof, including improved bone histology and mineral content (Declaration Exhibit "C", page 312, Figs. 3A-F); increased total body bone mineral content (TBBMC; *id.* at

page 312, Fig. 4B); fewer fractures; and achievement of normal median growth velocity (*id.* at page 312, Fig. 4A). These data demonstrate correlation between data obtained in the art-recognized murine OI model (Pereira et al., 1998, Proc. Natl. Acad. Sci. USA 95:1142-1147), and human *in vivo* data and demonstrate the applicability of results obtained using MSC cell therapy in an art recognized animal model to treatment of human disease.

The observations of Horwitz et al., 1999 (Exhibit "C"), were extended in Horwitz et al., 2001, Blood 97:1227-1231, a copy of which is attached hereto as Exhibit "D", which describes follow-up of the three OI patients for an additional 18-36 months beyond the original 6 month follow-up. The data disclosed in Horwitz et al., 2000, demonstrate that all three OI patients transplanted with normal donor MSCs in Horwitz et al., 1999 (Exhibit "C"), continued to demonstrate detectable improvement over controls in the clinical parameters assessed previously: increased growth depicted in terms of absolute bone growth (Exhibit "D", page 1229, Fig. 2A); increased growth rates which are similar to those of age-matched healthy children (*id.* at page 1229, Fig. 2B); all three patients had slightly greater TBBMC (mineral content) than normal weight-matched children (*id.* at page 1229, Fig. 3A-B).

The clinical data disclosed herein in Horwitz et al., 2000, demonstrate that, upon extended follow-up, MSC transplant recipients showed continued improvement. Further, the data establish that the effects of MSC-cell therapy are long lasting such that, *e.g.*, there is no problem with gene expression shutdown, and the like. Moreover, these data demonstrate that there is no detectable toxicity associated with MSC cell therapy.

Therefore, the data set forth in Exhibits "C" and "D" amply support claims reciting MSC cell therapy for human CNS diseases, conditions, or disorders since the results obtained in the art-recognized animal model demonstrate that successful cell therapy using MSCs in an art recognized model can be translated to successful treatment of the same disease in a human patient. The results of Horwitz et al., demonstrating that animal data using MSCs is correlated with human data using these cells, when combined with the data disclosed in Schwarz et al. (Exhibits "A" and "B"), demonstrating that recombinant MSCs can successfully treat a CNS disease, disorder or condition in an art-recognized animal model, support that the same result can be obtained in treating a human patient afflicted with Parkinson's disease using recombinant MSCs.

In addition, the data disclosed in Chen et al., 2001, Stroke 32:1005-10xx, attached hereto as Exhibit "E", which was published after the instant application and followed the teachings of the specification as filed, demonstrate that MSC cell therapy, as taught by applicants, provides a therapeutic benefit in an art-recognized model of stroke. More specifically, Chen et al., demonstrated that rats subjected to middle cerebral artery occlusion (MCAO), which is an art-recognized animal model for human stroke, demonstrated significant recovery of somatosensory behavior and Neurological Severity Score (NSS, which is a composite of motor, sensory, reflex, and balance tests) following transplantation with MSCs.

That is, $1x10^6$ (low-dose MSCs) or $3x10^6$ MSCs (high-dose MSCs) were injected into a rat tail vein either following or the in the absence of MCAO, and a battery of behavioral tests (*e.g.*, rotarod measurements, adhesive-removal somatosensory test, and NSS) were administered at various times after MCAO. The data disclosed in Chen et al., 2001, demonstrate that rats treated using low-dose MSCs showed no significant difference from non-transplanted rats in these tests. However, rats treated using high-dose MSCs demonstrated significant improvement in the various tests compared to control rats whether the MSCs were administered at 1, 7, or 14 days after ischemia (Exhibit "E", Table 1, and Figs. 1 and 2).

The data disclosed in Chen et al., also demonstrate that the donor MSCs were detected throughout the brain (*e.g.*, in multiple areas of the ipsilateral hemisphere, including cortexes, striatum of the ipsilateral hemisphere), with the vast majority of the cells detected in the ischemic core and its boundary zone and a few in the contralateral hemisphere (Exhibit "E", Table 2). These data are in accord with the observations of Schwarz et al. (Exhibit "C") that it appears that donor MSCs selectively migrate to the area of brain tissue damage.

Additionally, the data disclosed in Chen et al., demonstrate that some donor MSCs were reactive for the neuronal markers NeuN (neuronal nuclear antigen) and MAP-2 (microtobule-associated protein 2) (Exhibit "E", Figs. 3 and 4), supporting that MSCs differentiate into neural cells in the brain.

These data further support that, per the teachings of the specification as filed, cell therapy using MSCs can treat a CNS disease, disorder or condition, such as, but not limited to stroke/ischemia, as demonstrated in an art-recognized animal model of CNS disease, disorder, or condition. Further, as more fully set forth previously elsewhere herein, the data of Horwitz et al., demonstrate that results obtained MSC cell therapy in an art-recognized animal model of a

human disease, disorder, or condition correlate to treatment of the disease, disorder or condition in a human patient.

In addition, Li et al., 2001, Neurology 56:1666-1672, a copy of which is attached hereto as Exhibit "F", further demonstrates that MSC cell therapy provides a therapeutic treatment for stroke in an art-recognized animal model of human stroke. Specifically, rats were subjected to MCAO followed by intracarotid arterial injection of $2x10^6$ MSCs. The rats were then assessed using nerologic functional tests (adhesive-removal somatosensory test and modified NSS), before and at 1, 7, and 14 days post-MCAO. Additionally, the rat brains were examined using histologic and immunohistochemical assessment.

Li et al., demonstrate that MSC delivered to the carotid artery distribute over a wide area of the ischemic core and penumbra (Exhibit "F", Fig. 1, and text discussing same). Further, rats treated using MSC cell therapy demonstrated significant improvement in adhesive-removal tests and in modified NSS (*id.* at Figure 2). Also, the data disclosed in Li et al., demonstrate that the donor MSCs were found throughout the recipient brain and expressed GFAP and MAP-2 in the ischemic ipsilateral hemisphere and only expressed GFAP in the contralateral hemisphere.

Therefore, the data disclosed in Li et al., further demonstrate that, following the teachings of the invention, MSC cell therapy can successfully treat a CNS disease, disorder or condition, *e.g.*, stroke, as demonstrated using an art-recognized animal model for human stroke.

Further, data disclosed in Olson et al. (2001, In: Tissue Engineering for Therapeutic Use, pp. 21-36, Ikada and Oshima, eds., Elsevier Science), attached hereto as Exhibit "G", on which I am a co-author, demonstrate that MSC cell therapy is useful for treatment of spinal cord injury (SCI) in an art-recognized animal model for human SCI. That is, Olson et al., demonstrate that MSCs grafted to the intact or injured rat spinal cord survive and express neuronal markers such as NeuN (Exhibit "G", page 31, lines 7-10). Thus, in an art-recognized model of spinal cord injury, MSC therapy has been demonstrated to provide a therapeutic benefit in treating this CNS disease, disorder or condition.

Moreover, in Chopp et al. (2000, NeuroReport 11:3001-3005), a copy of which is attached hereto as Exhibit "H", MSCs were transplanted into the spinal cord in an art-recognized rat model of spinal cord injury. The data disclosed in Exhibit "H" demonstrate that MSCs injected into the rat spinal cord one week after a weight driven implant injury provide significant

improvement in functional outcome in transplanted animals. That is, significant improvement was detected using functional outcome measurements using the Basso-Beattie-Bresnehan (BBB) score, which is an art-recognized method of assessing SCI and improvement therefrom.

Chopp et al., demonstrate that rats injected with 2.5 x 10^5 MSCs at the epicenter of injury exhibited steady recovery that had not plateaued at five weeks and had significant improvement on BBB scores compared with control animals (*id.* at page 3003, Fig. 1). Further, Chopp et al., demonstrate that donor MSCs are present in the spinal cord and express neural protein markers (*e.g.*, NeuN) (*id.* at page 3004, Fig. 2). Therefore, Chopp et al., demonstrate successful treatment of spinal cord injury in an art-recognized animal model for human SCI. These data clearly demonstrate further reduction to practice of the invention with respect to spinal cord injury and amply support claims 9-18, as amended, reciting treatment of a human CNS disease, disorder or condition selected from the group consisting of Parkinson's disease, stroke, and spinal cord injury.

In summary, these data presented herein demonstrate that pursuant to the teachings of the specification as filed, the use of MSCs has been further reduced to practice. That is, the data described herein amply support that MSC cell and/or gene therapy in CNS diseases, disorders or conditions has been reduced to practice in art-recognized models of human CNS diseases. More specifically, the data discussed herein demonstrate treatment of Parkinson's disease, stroke, and spinal cord injury according to the methods disclosed in the specification as filed. Therefore, the data described herein demonstrate that a method for treating those diseases according to the methods disclosed in the specification as filed, is clearly enabled under 35 U.S.C. §112, first paragraph. Thus, Applicants respectfully submit that this rejection of claims 1-3 and 7-18, as amended, for lack of enablement, should be reconsidered and withdrawn.

Rejection of Claims 19 and 20, Under 35 U.S.C. § 103(a)

Claims 19 and 20 stand rejected under 35 U.S.C. § 103(a), as apparently being, in the Examiner's view, unpatentable over Pereira et al. (1995, Proc. Natl. Acad. Sci. USA 92:4857-4861) ("Pereira"), taken with Caplan (1991, J. Orthopaedic Research 9:641-650) ("Caplan"), Friedmann (1994, TIG 10:210-214) ("Friedmann"), and Prockop (1997, Science 276:71-74). Applicants, in response to the Office Action mailed May 24, 2000 (Paper No. 12), which Response was filed November 20, 2000, addressed the combination of Pereira, Caplan, and

Friedmann. The Examiner now adds Prockop to the combination and contends that Prockop teaches that directing differentiation of marrow-derived cells into a variety of cells is known in the art, that Friedmann teaches that marrow contains cells which can be directed to differentiate into CNS-associated cell types, and that Caplan (erroneously referred to as Eglitis et al.) and Pereira each teach that *in vivo* administration of marrow-derived cells results in differentiation of the cells into different lineages depending on which tissue is repopulated. The Examiner urges that one of skill in the art would have been motivated to establish cell culture conditions which allow the identification of bone marrow-derived cells and which would have allowed the identification of the microenvironment required to recapitulate the *in vivo* observations of Caplan (erroneously referred to as Eglitis) and Pereira.

The Examiner then reasons, based on the combined teachings of Pereira, Friedmann, Caplan, and Prockop, that the combination of these references renders methods of directing MSC differentiation *in vivo* by co-culturing the cells with differentiated cells, *e.g.*, astrocytes, obvious. Applicants respectfully submit that the combination of Pereira, Friedmann, and Caplan does not render claims 19 and 20, as amended, *prima facie* obvious under 35 U.S.C. §103(a), for the following reasons.

Preliminarily, claim 19, from which 20 depends, has been amended herein to recite that the cells differentiate into neural cells. Support for this amendment is found through out the specification as filed, commencing at page 27, lines 5-7. Thus, no new matter has been added by way of this amendment.

Further, Applicants respectfully point out that for the reasons set forth in Applicants' Response filed February 10, 2000, responding to Office Action mailed October 4, 1999 (Paper No. 6), Prockop is not a prior art reference for purposes of 35 U.S.C. §103(a). This is because Prockop, which was published on or about April 4, 1997, was published less that one year before the filing date of this application, *i.e.*, February 24, 1998.

Even assuming, *arguendo*, that Prockop is somehow a prior art reference for purposes of 35 U.S.C. §103(a), the combination of Pereira, Caplan, Friedmann and Prockop does not render claims 19 and 20, as amended, *prima facie*, obvious for the reasons set forth below.

Applicants respectfully submit that the three-prong test which must be met for a reference or a combination of references to establish a *prima facie* case of obviousness has not been satisfied in the instant matter. The MPEP states, in relevant part:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. MPEP § 2142.

None of these criteria have been met here.

Pereira, combined with Friedmann, Caplan, and Prockop does not teach or suggest all of the claim limitations. More specifically, claims 19 and 20, as amended herein, recite that the method of directing the differentiation of an isolated marrow stromal cell into neural cells comprises culturing the cell in the presence of a population of differentiated cells whereby the stromal cell differentiates into a cell of the same type as the cells it is co-cultured with. The combination of references urged by the Examiner does not teach these claim limitations. Indeed Pereira, Friedmann and Caplan do not mention any methods of *in vitro* culturing isolated stromal cells at all much less methods of co-culturing the cells with other cells in order to direct differentiation of the MSCs along a desired path. Pereira discloses only the administration of bone marrow-derived cells from a donor and tracing their subsequent fate in a recipient animal (*e.g.* where do the cells localize and what, if any, cell lineage-specific markers do they express). Therefore, Pereira merely discloses a passive observation and nowhere does Pereira teach or suggest that the differentiation process can be directed. Therefore, Pereira has nothing whatsoever to do with culturing isolated stromal cells or directing their differentiation in any way.

Similarly, Friedmann's discussion of stromal cells is limited to two paragraphs at page 212. In these paragraphs, Friedmann does not mention directing the differentiation of stromal cells at all but only discusses that since the mammalian CNS contains cells probably derived from bone marrow, bone marrow may be a useful source of cells which can be genetically engineered and introduced into the CNS to deliver a therapeutic gene product. There is no discussion in Friedmann concerning differentiation of stromal cells at all much less how this might be directed *in vitro*.

Caplan does not correct the deficiencies of Pereira and Friedmann since Caplan does not teach or suggest that MSCs can be directed to differentiate either *in vivo* or *in vitro*.

Indeed, Caplan teaches that differentiation from stem cell to final end phenotype is a *in vivo* complex process comprising a plethora of factors including paracrine and autocrine regulation. Thus, Caplan is, at the very most, merely an invitation to experiment to identify the various factors involved in stem cell differentiation, but there is nothing in Caplan which suggests that such trial-and-error transfection would be successful. Moreover, it is well-settled that an invitation to experiment is insufficient to support an obviousness rejection under 35 U.S.C. §103(a). Therefore, the Examiner's arguments at page 10 of the Office Action urging that these references would have "motivated" one skilled in the art "to establish cell culture conditions" to identify the "microenvironment required to recapitulate the *in vitro* observations of [Caplan] and Pereira", demonstrate that the combination of these references is, at most, an invitation for the artisan to attempt to arrive at the present invention.

Given the complexity of the differentiation of MSCs into various cell types in an *in vivo* milieu and the difficulties associated with adapting the *in vivo* observations to an *in vitro* cell culturing system, it would have been impossible for the skilled artisan to arrive at the present invention as recited in claims 19 and 20, without impermissible hindsight. That is, at the time the specification was filed, there was nothing in the combination of Pereira, Caplan, Friedmann and Prockop, to teach or suggest that MSCs could be directed to differentiate into any particular neural cell type, much less that such directed differentiation could be achieved *in vitro*, or, even more remotely, that differentiation could be directed by co-culturing the MSCs in a dish along with differentiated neural cells. Therefore, the teachings of Pereira and Caplan are, at the very most, an invitation to try, which is not sufficient under 35 U.S.C. §103(a), for purposes of rendering claims 19 and 20 *prima facie* obvious.

Further, there would have been no motivation to combine Pereira, Friedmann, Prockop, and Caplan to produce a method of directing stromal cell differentiation *in vitro* by co-culturing the stromal cell with differentiated cells. This is because Pereira, Friedmann, Caplan, and Prockop, alone or combined, does not teach or suggest directed differentiation of an isolated stromal cell and certainly does not teach or suggest how to direct differentiation *in vitro* by co-culturing with a substantially homogeneous population of cells. Moreover, Pereira and Friedman do not teach or suggest that stromal cell differentiation can be directed, much less how such differentiation would be accomplished in culture. In addition, Caplan has nothing whatsoever to do with differentiation of MSCs and does not teach or suggest that this complex process can be

accomplished *in vitro*. Thus, there was no motivation to combine these references to achieve the surprising results disclosed in the present application. Therefore, there could be no motivation to combine these references since the combination does not teach or suggest that stromal cells can be caused to differentiate into neural cells by co-culturing them with differentiated cells of a desired cell type.

In light of the foregoing arguments, it is clear that there was no reasonable expectation of success in combining the references to devise a method to direct differentiation of stromal cells by co-culturing them with a substantially homogenous population of differentiated cells of the desired cell type. That is, a person of ordinary skill in the art would not expect to succeed in directing differentiation of stromal cells by co-culturing the cells with a population of differentiated cells by combining references (*i.e.*, Pereira, Friedmann, Prockop, and Caplan) that have no suggestion or teaching as to how to direct differentiation of stromal cells *in vitro* using co-culturing them with other cells. As discussed previously elsewhere herein, Pereira, Friedmann, Prockop, and Caplan do not discuss directing stromal cell differentiation at all; instead, these references apparently note that bone marrow-derived cells implanted into a recipient animal can be found in certain tissues and express cell lineage-specific markers in those tissues. Nowhere in these references is there a teaching or suggestion that differentiation can be achieved by co-culturing stromal cells with a population of differentiated cells *in vitro*. Thus, there could be no reasonable expectation of success that combining Pereira, Friedmann, Prockop, and Caplan would result in the present invention.

For the reasons discussed above, the combination of Pereira with Friedmann, Prockop, and Caplan, cannot render claims 19 and 20, as amended, *prima facie* obvious under 35 U.S.C. § 103(a) and, therefore, the rejection should be reconsidered and withdrawn.

Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application has been either overcome or is now inapplicable, and that each of claims 1-3 and 7-20, is in condition for allowance. Reconsideration and allowance of each of these claims are respectfully requested at the earliest possible date.

Respectfully submitted,

DARWIN J. PROCKOP *ET AL.*

August 31, 2001
(Date)

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Enclosures: (Declaration of Darwin J. Prockop Pursuant to 37 C.F.R. §1.132 and Exhibits thereto; Request for Continued Examination and fee associated therewith)

1. (Amended) A method of treating a human patient having a disease, disorder or condition of the central nervous system, the method comprising obtaining a bone marrow sample from a human donor, isolating stromal cells from said bone marrow sample, and administering said isolated stromal cells to the central nervous system of said human patient, wherein the presence of said isolated stromal cells in said central nervous system effects treatment of said disease, disorder or condition, and further wherein said disease, disorder or condition is selected from the group consisting of Parkinson's disease, stroke, cerebral ischemia, and spinal cord injury.

19. (Amended) A method of directing the differentiation of an isolated stromal cell into a neural cell, comprising culturing said isolated stromal cell in the presence of a substantially homogeneous population of differentiated neural cells whereby said isolated stromal cell differentiates and acquires the phenotypic characteristics of said differentiated neural cells.